JAP9 Rec'd PCT/PTO 13 DEC 2089

PCT/USO4/18783.25022005

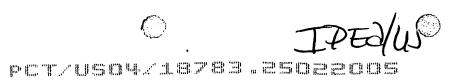
- 1. A synthetic, non-cytopathic negative-strand RNA virus replicon comprising
- a) a nucleotide sequence of said RNA virus, wherein the sequence of one or more structural genes is inactivated or deleted; and
- b) a nucleotide sequence encoding a selectable marker suitable for selection, wherein said sequence encoding a selectable marker is under the control of the RNA virus replication machinery and wherein the replicon, when introduced into a host cell, allows production of a stable culture of cells containing the replicon.
- 2. The replicon of claim 1, wherein said sequence encoding a selectable marker is a gene that confers resistance to an antibiotic.
- 3. The replicon of claim 2 wherein said gene is a bsd gene.
- 4. The replicon of claim 1, further comprising a sequence encoding a heterologous protein.
- 5. The replicon of claim 1 further comprising a reporter gene.
- 6. The replicon of claim 5, wherein said reporter gene is a gene encoding green fluorescent protein (GFP).
- 7. The replicon of claim 1 wherein said RNA virus is respiratory syncytial virus (RSV).
- 8. The replicon of claim 7, wherein the sequence encoding the F, G and SH glycoproteins is deleted.
- 9. The replicon of claim 8 wherein said sequence encoding a selectable marker is a gene that confers resistance to an antibiotic.
- 10. The replicon of claim 9, wherein said gene is a bsd gene.
- 11. The replicon of claim 10, further comprising a reporter gene.

PCT/USO4/18783 RESOBEDOS

- 12. The replicon of claim 11 wherein said reporter gene is a gene encoding GFP.
- 13. A cell line comprising the replicon of claim 1.
- 14. The replicon of claim 12, wherein said replicon is harbored in a cell line selected from the group consisting of BHK-RR-B51E (ATCC deposit number PTA-5257) and HeLa-RR-B51S (ATCC deposit number PTA-5258).
- 15. The replicon of claim 12, further comprising a sequence encoding a heterologous protein.
- 16. A cDNA of a non-cytopathic negative-strand RNA virus replicon comprising
- a) a nucleotide sequence complementary to the genome of said RNA virus, wherein the sequence encoding one or more structural genes is inactivated or deleted;
- b) a nucleotide sequence comprising a heterologous promoter sequence operatively linked to said sequence of a); and
- c) a nucleotide sequence comprising a gene encoding a selectable marker suitable for selection, wherein said gene is under the control of the RNA virus replication machinery and wherein the cDNA, when introduced into a host cell, allows production of a stable culture of cells containing the replicon.
- 17. The cDNA of claim 16, wherein said heterologous promoter sequence is selected from the group consisting of T7 polymerase promoter, cytomegalovirus immediate early promoter, SV40 early promoter and polymerase I promoter.
- 18. The cDNA of claim 16 wherein said promoter is a T7 polymerase promoter.
- 19. A replicon encoded by the cDNA of claim 16.
- 20. A method comprising
  - a) transfecting a cell line in culture with a polynucleotide comprising



- i) a DNA sequence complementary to a negative-strand RNA virus replicon, wherein the sequence encoding one or more structural proteins is inactivated or deleted;
- ii) a DNA sequence comprising a gene encoding a selectable marker protein suitable for selection;
  - b) culturing said cell line in vitro;
- c) selecting for cell populations displaying the phenotype conferred by said selectable marker, thereby producing a stable culture of cells containing the negative-strand RNA virus replicon; and
  - d) isolating RNA virus sequences from said cell populations of c).
- 21. The method of claim 20, wherein said selectable marker is a gene that confers resistance to an antibiotic.
- 22. The method of claim 21, wherein said antibiotic is blasticidin.
- 23. The method of claim 21, wherein said selecting of c) comprises culturing said cell line in a medium containing an antibiotic.
- 24. The method of claim 20, wherein said RNA virus is RSV.
- 25. The method of claim 24, wherein said RSV sequence comprises a mutation or deletion that renders the F, G and SH glycoproteins inoperative.
- 26. A method comprising
  - a) transfecting a cell line in culture with
- i) a DNA sequence complementary to a negative-strand RNA virus replicon, wherein the sequence encoding one or more glycoproteins is inactivated or deleted and wherein said sequence comprises a T7 polymerase promoter operatively linked to said sequence of I), and wherein said sequence further encodes a selectable marker; and
  - ii) a DNA sequence encoding a T7 polymerase;
  - b) culturing said cell line in vitro;



- c) selecting for cell populations displaying the phenotype conferred by said selectable marker thereby producing a stable culture of cells containing the negative-strand RNA virus replicon; and
  - d) isolating RNA virus sequences from said populations of c).
- 27. The method of claim 26, wherein step a) further comprises transfecting said cell line with support plasmids encoding viral proteins necessary for replication and mRNA synthesis.
- 28. A method for mobilizing a negative-strand RNA virus replicon comprising
- a) transfecting the cell line of claim 13 with a plasmid encoding a viral glycoprotein that allows virion formation;
  - b) culturing said cell line of a) in culture medium;
  - c) inoculating a fresh cell line with virions present in the culture medium of b).
- 29. The method of claim 28 wherein said viral glycoprotein that allows virion formation is a VSV G protein.
- 30. The method of claim 28, wherein said selectable marker is a gene that confers resistance to an antibiotic, said method further comprising
- d) culturing said inoculated cells of c) on medium containing the antibiotic; and
  - e) identifying replicon-expressing cells from the surviving cells.
- 31. A method comprising culturing a cell line containing the replicon of claim 4 in vitro to produce said heterologous protein.
- 32. A method for screening for antiviral agents comprising
  - a) contacting the cell line of claim 13 with a candidate agent, and
- b) testing for an increase or decrease in replication or activity of the RNA virus replicon relative to a control cell line harboring the same replicon, but which control cell line has not been contacted with the candidate agent.